

# MALARIA PARASITES IN THE PLACENTAL BLOOD

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During the months of July, August and September, 1924, the examination of twenty-six placentas for malaria parasites was carried out among native women in Freetown.

## EXAMINATION OF THE PLACENTA

The placenta arrived at the Laboratory at variable periods after delivery, sometimes within an hour and almost always within twelve hours. An incision was made through a cauterized area on the placenta, blood was withdrawn from the bottom of the cut by means of a pipette, films were spread, and stained either with Leishman's or Giemsa's stain. Malaria parasites were found in twelve, i.e., 46 per cent of the twenty-six cases.

## EXAMINATION OF MATERNAL PERIPHERAL BLOOD

The maternal peripheral blood was examined at the time of the birth in twenty-three of these cases, with the result that only four, i.e., 17 per cent. were found positive. No case of infection of the maternal peripheral blood was found in which the placental blood failed to show a much heavier infection.

Among the twenty-six cases there were four accidents (premature birth, etc.). In the twenty-two cases which had normal labour there were nine, i.e., 41 per cent., in which the placental blood showed parasites, whereas in the four cases which had abnormal labours there were three, i.e., 75 per cent., in which the placenta showed parasites.

### EXAMINATION OF THE UMBILICAL CORD

The umbilical cord was cut and films made from the blood in the vessels at two places, one as near as possible to the placenta and one about six inches from the placenta. In none of the twenty-six cases was infection of the umbilical cord blood found, in spite of the fact that in all cases where the placenta was heavily infected several thick films were examined, in addition to thin films; nor was infection found in the veins of the membranes.

### EXAMINATION OF CHILD

Films of the peripheral blood of the new-born child were made in twenty-four of the twenty-six cases; no infection was found.

In order to obtain information with regard to the possibility of congenital infection in the new-born child having been overlooked in the examination of cord and peripheral blood films, additional examinations were made.

1. In two cases of death of the child where the placenta of the mother was infected, an exhaustive search was made of smears of the heart blood, thymus, lungs, liver, spleen, bone marrow, and omentum with negative result.

In two similar cases where liver puncture alone was permitted, the smears proved negative.

2. Repeated examination of children (born of mothers with placenta infected) was carried out up to a week after birth and proved negative; thick as well as thin films being examined.
3. The blood of forty-one children aged one month or under was examined; infection was found in only one case, a child between three and four weeks old. Such a case as this cannot, for reasons given below, be classified as congenital.

Pezopoulos and Cardamatis (1907) drew attention to the heavy infection which may occur in maternal blood in the placenta at a time when the peripheral blood has only a few parasites. The preponderance of schizogony forms in the placental blood was noted by these observers. Their examinations of the umbilical cord blood, the peripheral blood of the child and organ smears led

them to conclude that the malaria parasite does not pass through the placenta from the maternal into the foetal circulation.

Clark (1915) examined films of the placental blood in a series of 400 cases, taking the blood from the maternal aspect of the placenta after the removal of clots. By this method he found *P. falciparum* in nineteen, i.e., 4·7 per cent. of the cases. Compared with this his findings of parasites in the maternal peripheral blood of the same cases taken at the end of labour were positive in only eight, i.e., 2 per cent. of the cases. In all the cases where the peripheral blood was positive, the placental blood showed a much heavier infection. Among the 400 cases there were forty-four accidents (abortion, still-birth, premature labour). In the 356 cases which had normal labour there were twelve, i.e., 3·4 per cent. in which the placental blood contained parasites, whereas in the forty-four cases which had abnormal labour there were seven cases, i.e., 16 per cent. in which the placental blood contained parasites.

In addition to the placental and maternal peripheral blood, Clark examined the blood in the umbilical cord. The cord was carefully cleaned, cut across and films were made from the foetal blood. In only one case were parasites found in blood from the cord; in this case the maternal peripheral and the placental bloods were both heavily infected, and the author considers it was due to the complication of an associated accident of pregnancy that the child had congenital infection.

#### CONGENITAL MALARIA

It is generally acknowledged that congenital malaria is a very rare condition. By congenital malaria is meant malaria which the unborn child acquires from the mother owing to failure of the barrier action of the placenta. The mechanism of the failure on the part of the placental barrier, i.e., whether it is due to the ability of exceptional parasites to penetrate through a healthy placenta, or to disease of, or accident to the placenta during pregnancy, does not here concern us.

The important point to decide is whether, in any given case, there is sufficient evidence to prove that it can only have been acquired owing to failure of this barrier action. Cases in which

parasites are found in the vessels of the umbilical cord at birth or in the peripheral blood or organs of the child at birth clearly belong to this category.

The more remote the period after birth at which parasites are found in the child the less justification have we for speaking of congenital malaria.

There are at least two definite and distinct ways in which a child not infected congenitally may become infected. The first way is by inoculation through abrasions of the skin in the process of delivery, where the maternal blood is mechanically inoculated into the child ; the second way is by the bites of infected mosquitos. Before any case of malaria can be established as congenital, it is clearly essential that the possibility of infection by either of these means should be absolutely excluded. This will be in many cases a difficult task, but the onus of proof is on those who claim cases as congenital. There are many cases standing in the literature to-day as cases of congenital malaria which are represented as congenital on altogether insufficient evidence.

It does not appear permissible to argue that the new-born child of a malarious mother is partially and temporarily tolerant and thus to explain the late development of symptoms and the late discovery of parasites in the child. There is little, if any, evidence to support this belief at present ; on the contrary, there does exist a small amount of definite evidence which tends to disprove it, namely, those cases in which parasites are present in the blood of the umbilical cord at birth, in the peripheral blood of the child at birth, and also some cases in which the parasites were found at the first examination of the child's blood a few days after birth. Such cases are all against the theory of partial immunity of the new-born child.

What is to be our criterion in judging whether a case is or is not congenital ? Our only available criterion is the minimum incubation period for the parasite, whether after inoculation of blood or after the bite of infected anophelines.

For example, in Yorke and Macfie's series of experimental infections with *Plasmodium vivax* by blood inoculation and mosquito bite infection, the shortest parasitic incubation found was after inoculation of infected blood, and was six days. We do not know whether, in the case of new-born children, this incubation period

might not be less. But accepting, for the present, six days as a minimum incubation period for *P. vivax*, it is not legitimate to assume to be congenital any case of infection with *P. vivax* in which parasites are first found more than six days after birth, unless other proof is supplied sufficient to show that the placental barrier has broken down.

When we consider the massive sporulating infections which the placental blood frequently shows (see Table I) and the absence of parasites in the cord and in the peripheral blood of the child, it appears certain that the walls of the villi are very efficient safeguards against the passage of merozoites even when these are present in enormous numbers. From the cases studied here, as well as from the literature dealing with congenital malaria, it appears that this condition is of great rarity.

#### THE PLACENTA AS AN INTERNAL ORGAN

In relapsing malaria, the parasites, in the intervals during which they cannot be discovered in the peripheral blood, are considered by most observers to be present in the internal organs. Of these, the spleen has always been the organ chiefly incriminated, the liver and bone marrow to a less extent have also been considered reservoirs. In the case of the spleen, however, it is known that even after removal of the organ, malaria may occur without re-infection. Obviously, in these cases, the spleen is not the only if even one of the chief reservoirs of the parasite. Acton, Knowles and Gupta (1923) punctured the spleen in fifteen cases and found splenic puncture to be 'a method of no diagnostic value in chronic malaria.'

In the present series an examination of the blood of the placenta has proved of striking value as a diagnostic of malaria. We recall that whereas examination of the peripheral blood revealed only 17 per cent. positive in twenty-three cases examined, the examination of the placenta revealed 46 per cent. positive in twenty-six cases.

#### NUMBERS AND STAGES OF PARASITE FOUND IN THE PLACENTA

In some of the blood films taken from infected placentas the number of parasites present is, as previously noted, quite remarkable. It might be approached rarely by the peripheral blood in fulminating cases of malaria, or more frequently by the capillary blood in

cerebral cases. We have never seen anything comparable to it in smears of spleen, liver, lung, kidney or bone marrow. Examination of the placenta at the time of child-birth, provided means of obtaining in women a malarial infection rate far higher than that obtained by even frequently repeated examinations of the peripheral blood.

The forms of parasites found in the placenta represent many stages not normally found in the peripheral blood. In all of the twelve placentas, parasites in the sporulating stage were present. In six cases sporulating forms were predominant, and in some of these, such forms constituted about 90 per cent. of all parasites found. In four of the other six cases there were about equal numbers of sporulating forms and young and medium-sized trophozoites. In two, the form of parasite present was almost exclusively the young trophozoite.

Double infection of cells was frequent, and in some instances two parasites in the same cell were sporulating at the same time.

#### COMPARISON OF INFECTION IN PERIPHERAL AND PLACENTAL BLOOD

The relative number of infected red blood corpuscles in the maternal peripheral and placental blood were determined in each of the four cases in which both the peripheral and placental blood contained parasites. Ten thousand red corpuscles in the peripheral blood were counted and five hundred in the placental. The results are shown in Table I.

TABLE I.

Showing the ratio of the numbers of parasites in the peripheral to those in the placental blood, and the percentage of infected cells in each, and the ratio thereof.

	Peripheral blood	Placental blood	Percentage of red cells infected		Ratio
			Peripheral	Placental	
Case I ... ..	1	1292	0.05	65.0	1 : 1300
Case II ... ..	1	44	0.05	2.5	1 : 50
Case III ... ..	1	20	0.03	0.6	1 : 20
Case IV ... ..	1	1395	0.04	56.0	1 : 1400

## CRESCENTS

It was notable that in none of the placenta infections were crescents seen, nor were they found in the peripheral blood where infected. When it is borne in mind that enormous numbers of parasites were examined in the placental blood in both thin and thick films, it seems reasonable to conclude that however favourable the conditions present in the placental blood may be for the development of asexual forms, they are, for some reason, unfavourable to the development of mature sexual forms.

That this absence of crescents was not a seasonal phenomenon affecting equally all cases is shown by the fact that, during the same period, crescents were present in 17 per cent. of ninety-six specimens of the peripheral blood examined in the children's clinic. The absence of crescents in the series of placentas examined here may be compared with the rarity of crescents recorded in the series of placentas examined by Clark.

**ENUMERATION OF MEROZOITES PRODUCED BY PARASITES  
IN PLACENTAL BLOOD**

Forms in which the process of sporulation was judged to be complete were chosen for counting; viz., those in which each merozoite was definitely separated from its neighbours. The maximum, minimum and average number of merozoites produced by the parasites of each case was thus determined. The highest count obtained was thirty-eight, but this was excluded owing to the possibility of its having a double infection of the red cell. The results are set out in Table II.

TABLE II.  
Numbers of merozoites produced by parasites in different cases.

	Total number of sporulating forms counted	Maximum number of merozoites produced	Minimum number of merozoites produced	Averages
Case I    ...    ...    ...	36	20	10	15
Case II    ...    ...    ...	36	26	15	19
Case III    ...    ...    ...	36	30	21	25
Case IV    ...    ...    ...	36	33	20	26

It will be observed that Cases III and IV, as contrasted with Cases I and II, show a far higher average number of merozoites per parasite when division is apparently completed, and also that the maximum and minimum figures obtained in each of these two cases are at a higher level. Attempts were made to ascertain by measurement the size of the merozoites, but this proved unsatisfactory. Even where the largest numbers were produced, e.g., thirty-three merozoites, there was much variation in the size of individuals, and such merozoites appeared on the average to be equal in size to those occurring in parasites producing a much smaller number. It appears possible that certain varieties of *P. falciparum* produce a larger number of merozoites than others; if this were so, it might have some bearing on the rapidity of the onset and the course of an attack.

Very little evidence is procurable as to the bio-chemical conditions prevailing in the placental blood; while it is generally admitted that the blood of the placenta differs in some respects both from the maternal peripheral and foetal bloods, the differences do not appear to have been accurately determined.

Regarded purely from the standpoint of the suitability for the development of *P. falciparum*, the placental blood appears to afford conditions which are not paralleled in the maternal peripheral blood. These conditions appear extremely favourable for the asexual phase of development, but not favourable for the sexual phase. The placental blood fulfills the following conditions known to be necessary for the culture *in vitro* of *P. falciparum*.

1. Stagnation of blood.
2. Limitation of oxygen.
3. Presence of glucose.

Yoshida and Ko (1920) have shown that in all types of malaria the blood sugar is increased during the pyrexial period; the maximum figure obtained was in infection with *P. falciparum*. Wells (1920) says glycogen is most abundant in the uterus at the time of child-birth and is abundant in the placenta.

It is interesting to recall the observation of Bass and Johns (1913) that the blood sugar of diabetics who have malaria renders the addition of dextrose to the culture medium unnecessary. It is perhaps relevant to remark that crescents have never been seen *in vitro* in media fulfilling the above-mentioned conditions.



## SUMMARY

1. Of twenty-six placentas of native women examined in Sierra Leone twelve, i.e., 46 per cent., were infected with *P. falciparum*.
2. The infection in many of these placentas was massive.
3. Examination of the peripheral blood of twenty-three of these cases revealed only four, i.e., 17 per cent., infected.
4. In the placental blood, sporulating parasites were numerous and also young and half-grown forms.
5. Definite differences were observed in the number of merozoites produced by the parasites of different cases ; these differences may influence the rapidity of onset and the course of the disease.
6. Crescents were never found in the placental blood in any of the cases examined.
7. No case of congenital malaria was encountered.
8. Some evidence is produced which suggests that malaria infection of the mother predisposes to accidents during pregnancy or at birth.

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